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(71) Applicant *(for all designated States except US)*: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : BROWN, Eugene, L. [US/US]; 1388 Walnut Street, Newton Highlands, MA 02161 (US).

(74) Agent: KAPINOS, Ellen, J.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).

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(57) Abstract

A composition and method for treating cancers characterized by over-expression of the *c-fms* proto-oncogene/M-CSF receptor protein are provided. The composition involves an M-CSF polypeptide cross-linked to a cytotoxic agent capable of crossing into the cytoplasm of the cell bearing the receptor and killing the cell.

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COMPOSITION AND METHOD FOR TREATING CANCERS CHARACTERIZED BY OVER-EXPRESSION OF THE C-FMS PROTO-ONCOGENE

The present invention refers generally to the treatment of a variety of cancers characterized by the over-expression of the protein receptor, c-fms. More specifically, the invention refers to a composition for such treatment including the M-CSF polypeptide linked to a cytotoxic agent.

BACKGROUND OF THE INVENTION

A variety of oncogenes have been associated with specific cancers. The oncogene fms has come under recent scrutiny as being related to breast, lung pancreatic, ovarian, renal, and possibly other carcinomas, including acute myelocytic leukemia (AML). See, e.g., D. J. Slamon et al, Science, 224:256-262 (1984); C. Walker et al, Proc. Natl. Acad. Sci., USA,:1804-1808 (April 1987). See also, J. H. Ohyashiki et al, Cancer Genet. Cytogenet., 25:341-350 (1987); H. D. Preisler et al, Cancer Research, 47:874-880 (Feb. 1987); C. W. Rettenmier et al, J. Cell. Biochem., 33:109-115 (1987); and R. Sacca et al, Proc. Natl. Acad. Sci. USA, 82:3331-3335 (1986). The product of the c-fms proto-oncogene is believed to be related to, and possibly identical with, a receptor of macrophage colony-stimulating factor (M-CSF). See, e.g., C. J. Sherr et al, Cell, 41:665-676 (1985);

There remains a need in the treatment of such cancers for therapeutic products capable of destroying the carcinoma cells without severely adversely affecting the patient otherwise.

BRIEF DESCRIPTION OF THE INVENTION

As one aspect of the invention there is provided a composition for treating cancers which are characterized by high level expression of the c-fms proto-oncogene/M-CSF

receptor gene. The composition includes M-CSF polypeptide (or the active fragment thereof) crosslinked to a cytotoxic agent, which is capable of crossing the membrane of the cell bearing the c-fms gene product/M-CSF receptor and acting in the cytoplasm to destroy the cell. Preferred cytotoxic agents include A and B chain toxins, A chain toxins and genetically engineered toxins.

In a further embodiment the composition may comprise a monoclonal antibody (or a portion thereof) to c-fms gene product/M-CSF receptor conjugated to a cytotoxic agent. This monoclonal moiety recognizes and binds to the c-fms gene product/M-CSF receptor. Antibody conjugates for the delivery of compounds to target sites and methods for preparing the same are known in the art. See e.g. U.S. Patent 4,671,958.

Still a further aspect of the invention involves a method for making the M-CSF/cytotoxic agent composition. The M-CSF and toxin may be linked by employing one or more heterofunctional or bifunctional protein cross linkers or by genetic fusion. The bifunctional cross-linkers are chosen to ensure that the M-CSF/toxin composition is stable while the composition is homing to the target cell. At the same time the crosslinker has to permit the release of the toxin portion after the M-CSF/toxin composition has entered the cell. See, e.g. Molecular Action of Toxins and Viruses, P. Cohen and S. van Heyningen, eds., Elsevier, New York, pp51-105 (1982).

As another aspect there is disclosed a method for treating cancers characterized by an over-expression of the c-fms proto- oncogene/M-CSF receptor gene. This method involves regionally administering to the in vivo site of such a cancer, the composition of the invention, or, alternatively, administering the composition in an ex vivo purging treatment of a mixture of cells. The composition acts by attaching to the c-fms protein on the carcinoma and delivering the toxin through the cell membrane, where the

toxin destroys the cell. Among such receptor over-expressing cancers are acute myelocytic leukemia, ovarian carcinoma, lung carcinoma, and those recited above.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description of the invention, including illustrative examples of the practice thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a DNA and amino acid sequence for an M-CSF polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The therapeutic composition of the invention is a conjugate of M-CSF, which is capable of binding to the <u>c-fms</u> proto-oncogene/M-CSF receptor gene product on certain cancer cells, and a cytotoxic agent capable of being transported through the cell membrane and acting in the cytoplasm to destroy the cell.

The M-CSF for use in the present invention may be recovered from natural sources and purified. (See e.g, UK Patent 2,016,477 and PCT published application WO86/04587). Alternatively, the M-CSF may be produced recombinantly. One possible recombinant M-CSF polypeptide useful in the present invention has been described in PCT published application WO86/04607. Another M-CSF polypeptide is described in co-pending, co-owned US patent application SN940,362 and in G. G. Wong et al, <u>Science</u>, <u>235</u>:1504-1508 (1987). The amino acid and DNA sequence of the M-CSF described therein is presented hereto in Fig. 1. Other forms of M-CSF bearing the active site thereof may also be employed in this composition, including synthetically produced polypeptides or polypeptides modified by recombinant means.

The term "M-CSF" is herein defined as including the naturally occurring human polypeptide M-CSF and naturally-

occurring allelic variations of the polypeptide. Allelic variations are naturally-occurring base changes in the species population which may or may not result in an amino acid change in a polypeptide or protein. Additionally included in this definition are both recombinant and synthetic versions of the polypeptide M-CSF, which may contain induced modifications in the peptide and DNA sequences thereof.

For example, the M-CSF polypeptide in the composition of the present invention may be characterized by a peptide sequence the same as or substantially homologous to the amino acid sequence illustrated in Fig. 1. These sequences may be encoded by the DNA sequence depicted in Fig. 1 or sequences containing allelic variations in base or amino acid sequence or deliberately modified structures coding for polypeptides with M-CSF biological properties.

Synthetic M-CSF proteins for use in the composition of the present invention may wholly or partially duplicate continuous sequences of the amino acid residues of Fig. 1. These sequences, by virtue of sharing structural and conformational characteristics with M-CSF polypeptides, e.g., the active site of the polypeptide of Fig. 1, may also possess M-CSF biological properties. Thus synthetic or recombinant polypeptides or fragments thereof may also be employed as biological or immunological equivalents for M-CSF polypeptides in the composition and methods of the present invention.

M-CSF, as used in the present invention also includes factors encoded by sequences similar to Fig. 1, but into which modifications are naturally provided or deliberately engineered. Modifications in the peptide or sequence of M-CSF can be made by one skilled in the art using known techniques. Specific modifications of interest in the M-CSF related sequences may include the replacement of one or more of the nine cysteine residues in the coding sequence with

other amino acids. Preferably several cysteines in each sequence are replaced with another amino acid, e.g. serine, to eliminate the disulfide bridges at those points in the protein. For example, lysine at amino acid position 163 (Fig. 1) could be deleted or substituted with another amino acid in order to eliminate the sensitivity of this region of M-CSF to trypsin-like proteases. Mutagenic techniques for such replacement are well known to one skilled in the art. [See, e.g., United States patent 4,518,584.]

Other specific mutations of the sequence of M-CSF described herein involve modifications of one or more of the glycosylation sites in the sequence. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one, two, three or all of the asparagine-linked glycosylation recognition sites present in the sequence of M-CSF. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Modification and variation of the types of oligosaccharides which attach to the O or N-linked glycosylation sites can occur by production of the sequence in either mammalian, bacterial, yeast or insect cells. Such modifications in the proteins are also encompassed by the term M-CSF.

Yet further modifications of M-CSF polypeptides may employ sequences which are designed for improved pharmacokinetics, by, e.g., association with polyethylene glycol. Alternatively, the last 25 to 35 amino acids of the

mature protein can be eliminated by appropriate gene deletion techniques to provide another form of M-CSF for use in the present invention. Such a deleted M-CSF may have use in genetic fusion to a cytotoxic agent. Amino acid residues 464 to 485 comprise a potential hydrophobic membrane-penetrating region. An M-CSF molecule that contains this sequence may desirably be employed in the composition of the invention, because these residues may embed the conjugate in the cell membrane, thereby aiding in the transfer of the cytotoxic agent into the cytosol.

An exemplary DNA sequence for the production of various M-CSF peptides have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD. The cDNA sequence illustrated in Fig. 1 below in vector p3ACSF-69, included in E. coli HB101 has been deposited on April 16, 1986 and given accession number ATCC 67092. This deposit was made under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the Regulations thereunder (Budapest Treaty).

The cytotoxic agent linked to the M-CSF polypeptide is preferably a toxin or chemical agent which is capable of acting in the cytoplasm. Toxins may be employed which have a translocation property to move it through the cell membrane and a cytolytic domain, which provides its killing ability. One preferable class of toxins well-suited for this composition consists of two functionally different parts, termed A and B, which are connected by a disulfide bond. The A chain portion contains the enzymatic activity that enters the cytosol and kills the cell. The B chain moiety is responsible for binding of the toxin to the cell and presumably contains a domain that aids the A chain in crossing the cell membrane. Exemplary toxins for such use include native or genetically engineered ricin, abrin, modeccin, viscumin, Pseudomonas aeruginosa exotoxin,

Diphtheria toxin, Cholera toxin, Shigella toxin and E. coli heat labile toxin. The toxin portion of a conjugate prepared according to the invention can consist of the cytotoxic A chain portion only, the native holotoxin, or an engineered holotoxin, i.e., a toxin lacking its lectin binding property.

Other toxins which have only a single chain (an A chain portion) may also be employed. Examples of these toxins are ribosome inactivating proteins, such as pokeweed antiviral protein and gelonin. See, L. Barbieri et al, Cancer Surveys, 1:489-520 (1982) for a more complete list of ribosome inactivating proteins.

Mutant toxins or genetically engineered toxins may also be employed. Additionally microbially produced cytotoxic agents, and other non-protein organic molecules may be used as cytotoxic agents. The M-CSF ligand can also be linked to cytotoxic drugs, such as anthracyclines, e.g., doxorubicin, daunomycin, and the vinca alkaloids, such as, vindesine, vinblastine, vincristine. Methotrexate and its derivatives may also be employed as cytotoxic agents. More effective agents are those in which many molecules (between 5 to 50) of the drug are linked to the M-CSF through a polymer carrier, e.g., dextran. Bonds linking the drug to the carrier should be cleavable by the chemical environment inside the cell.

The M-CSF and a cytotoxic agent may be linked in a variety of ways. One way of linking these components is by employing one or more standard bifunctional protein crosslinkers, such as succinimidyl 3-(2-pyridyldithio)propionate (SPDP) or succinimidyl acetyl-thiopropriate (SATP). These crosslinkers form stable disulfide bonds between the M-CSF and toxin, or other cytotoxic agent, and yet are capable of releasing the toxin portion of the composition inside the cell, due to cleavage of the disulfide bonds by chemicals inside the cell, e.g., intracellular glutathione. These linking methods are known to those skilled in the art. See, e.g., J. Carlsson et al,

Biochem. J., 173:723-737 (1978) and N. Fujii et al, Chem. Pharm. Bull., 33:362-367 (1985). See also, A. J. Cumber et al, Methods Enzymol., 112:207-225 (1985) for other general methods for conjugating toxins to proteins.

For example, one method according to the invention involves making a M-CSF-toxin composition, using a toxin having both and A and B chain. The method involves the steps of:

(a) reacting the M-CSF with sufficient crosslinker to introduce between 1 to 6 reactive groups per molecule of M-CSF. A sufficient amount of crosslinker which can be used for this purpose is between approximately 6 to 50 moles of crosslinker per mole of M-CSF dimer.

(b) reacting a toxic protein having A and B chain subunits connected by at least one disulfide bond with a conventional reducing agent, thereby liberating the chains from each other.

(c) reacting the derivitized M-CSF of step (a) with the liberated A chain subunit of the reduced toxin; and

(d) separating from the reaction mixture conjugates comprising M-CSF linked by disulfide bonds to A chain subunits.

One exemplary growth factor/toxin conjugate is prepared by this method, modifying M-CSF with SPDP, followed by conjugation of ricin A chain toxin via a disulfide bond.

Another method for making the compositions of the present invention involves the following steps:

(a) reacting the M-CSF with sufficient crosslinker to introduce between 1 to 6 reactive groups per molecule of M-CSF;

(b) reacting the derivatized M-CSF of step (a) with a holotoxin having A and B subunits attached by at least one disulfide bond, the holotoxin being functionalized with a protein crosslinker which is preferably attached to the B subunit; and

(c) separating a conjugate formed by M-CSF becoming attached to the B subunit from free M-CSF and toxin in the reaction mixture.

Another manner of linking the components of the composition of the present invention is by a genetic fusion method. See, for example, United States Patent 4,675,382.

The compositions of the present invention containing both M-CSF and a toxin can be employed in methods for treating cancers characterized by over-expression of the c-fms proto-oncogene/M-CSF receptor gene. Among such cancers are acute myelocytic leukemia, ovarian cancer, breast cancer, lung cancer, pancreatic cancer and renal cancer. The composition of the invention operates by the targeting of the c-fms proto-oncogene by the M-CSF portion of the composition. Once attached to this receptor, the M-CSF molecule aids in transporting the cytotoxic agent through the cell membrane and into the cytosol. Inside the cell, the bonds linking the cytotoxic agent to the M-CSF are cleaved by chemicals naturally within the cell and the agent is released to kill the cancer cell.

The composition of the present invention can be administered in a variety of ways including systemically, locally or regionally. Desirably the composition is administered regionally *in vivo*, to the site of the carcinoma. For example, it can be administered intraperitonially, if desired, to contain its distribution to the peritoneum for use in treating a suitable cancer, e.g., ovarian cancers. Similarly for treating lung cancers, the composition could be delivered in the form of an inhalant. If desirable, the composition may be administered subcutaneously, such as bathing effected tissue after surgical removal of a tumor e.g., for breast cancers. The composition may preferably be administered intravesically for instance into the bladder. Additionally, the composition can be employed in *ex vivo* applications, such as "purging" of a

mixture of cells removed from a patient, for patients having a systemic cancer which is not appropriate for regional application. The treatment of patients with acute myelocytic leukemia, for example, could involve removal of bone marrow cells from the body. These cells are then treated outside the body with the composition of the present invention to destroy a subset of these cells which are overexpressing the c-fms proto-oncogene. The "purged" cells are then reintroduced into the patient. The M-CSF/toxin composition of the invention can thereby serve as a purging agent to destroy the leukemic cells in the bone marrow of AML patients about to undergo autologous bone marrow transplantation. Other *ex vivo* purging treatments may also employ the composition of the invention.

The therapeutic composition for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

The dosage regimen involved in treating the patient with the composition according to this invention will be determined by the attending physician considering various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of adminis- tration and other clinical factors. Additionally, the mode of administration could effect the dosage, e.g., *ex vivo* or *in vivo*. Generally, the daily regimen should be in the range of 2 to 2000 micrograms of polypeptide per kilogram of body weight.

The following examples illustrate the production of the M-CSF polypeptide and the construction of an M-CSF/toxin conjugate of the present invention.

EXAMPLE 1

Recombinant Production of M-CSF

To express the recombinant M-CSF polypeptide by recombinant means, the DNA encoding the polypeptide is transferred into an appropriate expression vector and introduced into selected host cells by conventional genetic engineering techniques.

Mammalian cell expression vectors for production of M-CSF, such as p3ACSF-69, may be synthesized by techniques well known to those skilled in this art. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. See, Kaufman et al, J. Mol. Biol., 159:511-521 (1982); and Kaufman, Proc. Natl. Acad. Sci., U.S.A., 82:689-693 (1985). Suitable cells or cell lines for the expression of these recombinant M-CSF proteins may be Chinese hamster ovary cells (CHO), monkey COS-1 cells or CV-1 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Other exemplary mammalian host cells include particularly primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells need not be genotypically deficient in the selection gene so long as the selection gene is dominantly acting. For stable integration of the vector DNA, and for subsequent amplification of the integrated vector DNA, both by conventional methods, CHO cells may be employed. Other suitable mammalian cell lines include but are not limited to, HeLa, mouse L-929 cells, 3T3 lines

derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Stable transformants are then screened for expression of the product by standard immunological or enzymatic assays. The presence of the DNA encoding the variant proteins may be detected by standard procedures such as Southern blotting. Transient expression of the DNA encoding the variants during the several days after introduction of the expression vector DNA into suitable host cells such as COS-1 monkey cells is measured without selection by activity or immunologic assay of the proteins in the culture medium. The transformation of these vectors into appropriate host cells can result in expression of the M-CSF.

Similarly, one skilled in the art could manipulate the sequence of Fig. 1 by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression of M-CSF by bacterial cells. The DNA encoding the factor may be further modified to contain different codons for bacterial expression as is known in the art. Preferably the sequence is operatively linked in-frame to a nucleotide sequence encoding a secretory leader polypeptide permitting bacterial expression, secretion and processing of the mature variant protein, also as is known in the art. The compounds expressed in bacterial host cells may then be recovered, purified, and/or characterized with respect to physicochemical, biochemical and/or clinical parameters, all by known methods. For example, the M-CSF coding sequence could be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and the factor expressed thereby. The various strains of E. coli (e.g., HB101, MC1061) are well-known as

host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method. For a strategy for producing extracellular expression of such factors in bacterial cells, see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See, e.g., procedures described in published European patent application 155,476] for expression in insect cells. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of M-CSF by yeast cells. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides useful in the invention. [See, e.g., procedures described in published PCT application WO 86 00639 and European patent application EP 123,289.]

EXAMPLE 2

An M-CSF Toxin Conjugate

For construction of an M-CSF toxin conjugate according to the invention, the growth factor M-CSF was produced in mammalian cells as described in pending U. S. patent SN940,362, the disclosures of which are incorporated by reference herein, and G. G. Wong et al, Science, 235 supra. M-CSF (5 mg, 55 nmoles) in 0.1M $NaHCO_3$ (20 ml) was reacted with a 20-fold molar excess of SPDP in ethanol. The reaction was allowed to proceed for five hours at 4 degrees Celsius to introduce approximately four to six sulfhydryl groups per molecule of M-CSF dimer. After removal of excess SPDP the derivatized growth factor was reacted with ricin A (15 mg, 500 nmoles), obtained from a commercial source, in 50mM NaH_2PO_4 p. 117.5/OIM NaCL. The disulfide bond was allowed

to form overnight at 4 degrees Celsius. The resulting M-CSF-ricin A chain conjugate was separated from excess ricin A chain by gel filtration on a Sepherogel™ TSK-3000 high pressure liquid chromatography column to give a mixture of conjugate and M-CSF (7.5 mg). After two passages through a column of Blue Sepharose developed with a gradient of NaCl, as described by P. P. Knowles and P. E. Thorpe, Anal. Biochem., 160: 440-443 (1987), the conjugate (720 mg) was obtained in a form free of M-CSF and consisted mainly of a species with one ricin A chain per M-CSF dimer.

EXAMPLE 3

In Vitro Cytotoxicity of M-CSF Toxin Conjugate

A level of toxicity and specificity for the M-CSF/ricin A chain conjugate was determined in a standard soft agar clonogenic assay in a manner similar to that described by Strong et al, Blood, 65: 627-635 (1985) with the NIH 3T3 and NIH 3T3-c-fms cell lines. The latter line which has been described by M. F. Roussel et al, Nature, 325: 549-552 (1987), is M-CSF receptor positive. Each cell line was mixed with either conjugate or medium without conjugate (control) in agarose and thin layered into Petri dishes. After incubation at 37°C in standard CO_2 atmosphere for a period of 14 days, the number of colonies in each dish was counted visually. The NIH 3T3-c-fms cells control dishes which did not receive the conjugate showed 103 colonies per dish while the same cells treated with conjugate at a concentration of 4 X 10^{-8}M gave only 3 colonies. The NIH 3T3 cells, treated with conjugate and untreated control cells mixed with medium gave 76 and 78 colonies per dish, respectively.

EXAMPLE 4

Ex Vivo Assay of M-CSF Toxin Conjugate

The efficacy of the M-CSF/ricin A chain conjugate for ex vivo bone marrow purging is tested in a manner analogous to

that described by Strong et al, supra. M1 myeloid leukemic cells (10^3) which may be obtained from the American Type Culture Collection, Rockville, Maryland, (ATCC TIB 192) are added to murine bone marrow cells (10^5) and then treated with the M-CSF/ricin A conjugate in the 10^{-7} - 10^{-12}M range for approximately 4 hours at 37C. The percent survival of the leukemic cells as well as the monopotent and pluripotent bone marrow progenitor cells is determined with a standard colony formation assay, T.R. Bradley and D. Metcalf, Aust. J. Exp. Biol. Med. Sci., 44: 287 (1966) to measure the efficacy and specificity, respectively.

Numerous modifications may be made by one skilled in the art to the methods and components of the present invention in view of the disclosure herein. Such modifications are believed to be encompassed in the appended claims.

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page ______, line ______ of the description [1]

A. IDENTIFICATION OF DEPOSIT [1]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

American Type Culture Collection

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852 USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
p3ACSF-69	67092	6/15	16 April 1986

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(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

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Form PCT/RO 134 (January 1981)

WHAT IS CLAIMED IS:

1. A therapeutic composition for treating carcinoma characterized by over-expression of the c-fms proto-oncogene/M-CSF receptor gene comprising a M-CSF polypeptide conjugated to a cytotoxic agent and pharmaceutical carrier therefor.

2. The composition according to Claim 1, wherein said cytotoxic agent is a toxin selected from the group comprising double-chain ricin, ricin A chain, abrin, abrin A chain, modeccin and modeccin A chain, Pseudomonas aeruginosa exotoxin, Cholera toxin, Shigella toxin, E. coli heat labile toxin and Diphtheria toxin, mutant toxins thereof, and recombinant versions thereof.

3. The composition according to claim 1 wherein said cytotoxic agent is selected from the group consisting of ribosome-inactivating proteins, pokeweed antiviral protein and gelonin, mutant toxins thereof, and recombinant versions thereof.

4. The composition according to claim 1 wherein said cytotoxic agent is selected from the group consisting of anthracyclines, doxorubicin, daunomycin, vinca alkaloids, vindesine, vinblastine, vincristine, methotrexate and derivatives thereof.

5. The composition according to claim 1 where said M-CSF polypeptide is conjugated to said cytotoxic agent by a heterofunctional protein cross linking agent.

6. The composition according to claim 5 where said cross linking agent is selected from the group consisting of succinimidyl 3-(2-pyridyldithio)propionate) or succinimidyl

acetylthiopropriate.

7. The composition according to claim 1 comprising M-CSF conjugated through SPDP to a full ricin molecule.

8. A method for treating cancers characterized by an overexpression of the _c-fms_ proto-oncogene/M-CSF receptor protein, comprising regionally administering _in vivo_ to the site of said cancer a composition comprising M-CSF linked to a cytotoxic agent capable of crossing the membrane of the cell bearing said receptor and entering and killing the cell.

9. A method for treating cancers characterized by an overexpression of the _c-fms_ proto-oncogene/M-CSF receptor protein, comprising _ex vivo_ purging of a mixture of cells removed from a patient, said mixture containing said cancer cells, with a composition comprising M-CSF linked to a cytotoxic agent capable of crossing the membrane of the cell bearing said receptor and entering and killing the cancer cells.

10. A composition for treating carcinoma characterized by over-expression of _c-fms_ proto-oncogene/M-CSF receptor gene comprising a monoclonal antibody to c-fms gene product/M-CSF receptor said monoclonal antibody conjugated to a cytotoxic agent and pharmaceutical carrier therefor.

Figure 1

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        10         20         30         40         50         60         70
CCTGGGTCCT CTCGGCGCCA GAGCCGCTCT CCGCATCCCA GGACAGCGGT GCGGCCCTCG GCCGGGGCGC

        80         90        100        110        120        130        140
CCACTCCGCA GCAGCCAGCG AGCGAGCGAG CGAGCGAGGG CGGCCGACGC GCCCGGCCGG GACCCAGCTG

     (-32)          160                 175                 190
CCCGT ATG ACC GCG CCG GGC GCC GCC GGG CGC TGC CCT CCC ACG ACA TGG CTG
      MET Thr Ala Pro Gly Ala Ala Gly Arg Cys Pro Pro Thr Thr Trp Leu

            205                 220                 235         (1)
GGC TCC CTG CTG TTG TTG GTC TGT CTC CTG GCG AGC AGG AGT ATC ACC GAG GAG
Gly Ser Leu Leu Leu Leu Val Cys Leu Leu Ala Ser Arg Ser Ile Thr Glu Glu

250                 265                 280                 295
GTG TCG GAG TAC TGT AGC CAC ATG ATT GGG AGT GGA CAC CTG CAG TCT CTG CAG
Val Ser Glu Tyr Cys Ser His MET Ile Gly Ser Gly His Leu Gln Ser Leu Gln

        310                 325                 340                 355
CGG CTG ATT GAC AGT CAG ATG GAG ACC TCG TGC CAA ATT ACA TTT GAG TTT GTA
Arg Leu Ile Asp Ser Gln MET Glu Thr Ser Cys Gln Ile Thr Phe Glu Phe Val

                370                 385                 400
GAC CAG GAA CAG TTG AAA GAT CCA GTG TGC TAC CTT AAG AAG GCA TTT CTC CTG
Asp Gln Glu Gln Leu Lys Asp Pro Val Cys Tyr Leu Lys Lys Ala Phe Leu Leu

    415                 430                 445                 460
GTA CAA GAC ATA ATG GAG GAC ACC ATG CGC TTC AGA GAT AAC ACC CCC AAT GCC
Val Gln Asp Ile MET Glu Asp Thr MET Arg Phe Arg Asp Asn Thr Pro Asn Ala

            475                 490                 505
ATC GCC ATT GTG CAG CTG CAG GAA CTC TCT TTG AGG CTG AAG AGC TGC TTC ACC
Ile Ala Ile Val Gln Leu Gln Glu Leu Ser Leu Arg Leu Lys Ser Cys Phe Thr

520                 535                 550                 565
AAG GAT TAT GAA GAG CAT GAC AAG GCC TGC GTC CGA ACT TTC TAT GAG ACA CCT
Lys Asp Tyr Glu Glu His Asp Lys Ala Cys Val Arg Thr Phe Tyr Glu Thr Pro

        580                 595            (122)610                 625
CTC CAG TTG CTG GAG AAG GTC AAG AAT GTC TTT AAT GAA ACA AAG AAT CTC CTT
Leu Gln Leu Leu Glu Lys Val Lys Asn Val Phe Asn Glu Thr Lys Asn Leu Leu

                640                 655                 670
GAC AAG GAC TGG AAT ATT TTC AGC AAG AAC TGC AAC AAC AGC TTT GCT GAA TGC
Asp Lys Asp Trp Asn Ile Phe Ser Lys Asn Cys Asn Asn Ser Phe Ala Glu Cys
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Figure 1 (Con't)

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    685                      700                      715                      730
TCC AGC CAA GAT GTG GTG ACC AAG CCT GAT TGC AAC TGC CTG TAC CCC AAA GCC
Ser Ser Gln Asp Val Val Thr Lys Pro Asp Cys Asn Cys Leu Tyr Pro Lys Ala

            745                      760                      775
ATC CCT AGC AGT GAC CCG GCC TCT GTC TCC CCT CAT CAG CCC CTC GCC CCC TCC
Ile Pro Ser Ser Asp Pro Ala Ser Val Ser Pro His Gln Pro Leu Ala Pro Ser

790                     805(189)                 820                      835
ATG GCC CCT GTG GCT GGC TTG ACC TGG GAG GAC TCT GAG GGA ACT GAG GGC AGC
MET Ala Pro Val Ala Gly Leu Thr Trp Glu Asp Ser Glu Gly Thr Glu Gly Ser

        850                      865                      880                      895
TCC CTC TTG CCT GGT GAG CAG CCC CTG CAC ACA GTG GAT CCA GGC AGT GCC AAG
Ser Leu Leu Pro Gly Glu Gln Pro Leu His Thr Val Asp Pro Gly Ser Ala Lys

                910                      925                      940
CAG CGG CCA CCC AGG AGC ACC TGC CAG AGC TTT GAG CCG CCA GAG ACC CCA GTT
Gln Arg Pro Pro Arg Ser Thr Cys Gln Ser Phe Glu Pro Pro Glu Thr Pro Val

    955                      970                      985                      1000
GTC AAG GAC AGC ACC ATC GGT GGC TCA CCA CAG CCT CGC CCC TCT GTC GGG GCC
Val Lys Asp Ser Thr Ile Gly Gly Ser Pro Gln Pro Arg Pro Ser Val Gly Ala

        1015                     1030                     1045
TTC AAC CCC GGG ATG GAG GAT ATT CTT GAC TCT GCA ATG GGC ACT AAT TGG GTC
Phe Asn Pro Gly MET Glu Asp Ile Leu Asp Ser Ala MET Gly Thr Asn Trp Val

1060                    1075                     1090                     1105
CCA GAA GAA GCC TCT GGA GAG GCC AGT GAG ATT CCC GTA CCC CAA GGG ACA GAG
Pro Glu Glu Ala Ser Gly Glu Ala Ser Glu Ile Pro Val Pro Gln Gly Thr Glu

    1120                     1135                     1150                     1165
CTT TCC CCC TCC AGG CCA GGA GGG GGC AGC ATG CAG ACA GAG CCC GCC AGA CCC
Leu Ser Pro Ser Arg Pro Gly Gly Gly Ser MET Gln Thr Glu Pro Ala Arg Pro

                1180                     1195                     1210
AGC AAC TTC CTC TCA GCA TCT TCT CCA CTC CCT GCA TCA GCA AAG GGC CAA CAG
Ser Asn Phe Leu Ser Ala Ser Ser Pro Leu Pro Ala Ser Ala Lys Gly Gln Gln

    1225                     1240                     1255                     1270
CCG GCA GAT GTA ACT GGT ACA GCC TTG CCC AGG GTG GGC CCC GTG AGG CCC ACT
Pro Ala Asp Val Thr Gly Thr Ala Leu Pro Arg Val Gly Pro Val Arg Pro Thr

            1285                     1300                     1315
GGC CAG GAC TGG AAT CAC ACC CCC CAG AAG ACA GAC CAT CCA TCT GCC CTG CTC
Gly Gln Asp Trp Asn His Thr Pro Gln Lys Thr Asp His Pro Ser Ala Leu Leu
```

Figure 1 (Con't)

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1330                    1345                     1360                     1375
AGA GAC CCC CCG GAG CCA GGC TCT CCC AGG ATC TCA TCA CTG CGC CCC CAG GGC
Arg Asp Pro Pro Glu Pro Gly Ser Pro Arg Ile Ser Ser Leu Arg Pro Gln Gly

        1390                     1405                     1420                     1435
CTC AGC AAC CCC TCC ACC CTC TCT GCT CAG CCA CAG CTT TCC AGA AGC CAC TCC
Leu Ser Asn Pro Ser Thr Leu Ser Ala Gln Pro Gln Leu Ser Arg Ser His Ser

                  1450                     1465                     1480
TCG GGC AGC GTG CTG CCC CTT GGG GAG CTG GAG GGC AGG AGG AGC ACC AGG GAT
Ser Gly Ser Val Leu Pro Leu Gly Glu Leu Glu Gly Arg Arg Ser Thr Arg Asp

    1495                     1510                     1525                     1540
CGG AGG AGC CCC GCA GAG CCA GAA GGA GGA CCA GCA AGT GAA GGG GCA GCC AGG
Arg Arg Ser Pro Ala Glu Pro Glu Gly Gly Pro Ala Ser Glu Gly Ala Ala Arg

              1555                     1570                     1585
CCC CTG CCC CGT TTT AAC TCC GTT CCT TTG ACT GAC ACA GGC CAT GAG AGG CAG
Pro Leu Pro Arg Phe Asn Ser Val Pro Leu Thr Asp Thr Gly His Glu Arg Gln

1600                    1615                     1630                     1645
TCC GAG GGA TCC TCC AGC CCG CAG CTC CAG GAG TCT GTC TTC CAC CTG CTG GTG
Ser Glu Gly Ser Ser Ser Pro Gln Leu Gln Glu Ser Val Phe His Leu Leu Val

        1660                     1675                     1690                     1705
CCC AGT GTC ATC CTG GTC TTG CTG GCT GTC GGA GGC CTC TTG TTC TAC AGG TGG
Pro Ser Val Ile Leu Val Leu Leu Ala Val Gly Gly Leu Leu Phe Tyr Arg Trp

                  1720                     1735                     1750
AGG CGG CGG AGC CAT CAA GAG CCT CAG AGA GCG GAT TCT CCC TTG GAG CAA CCA
Arg Arg Arg Ser His Gln Glu Pro Gln Arg Ala Asp Ser Pro Leu Glu Gln Pro

    1765                     1780                     1795                     1817
GAG GGC AGC CCC CTG ACT CAG GAT GAC AGA CAG GTG GAA CTG CCA GTG TAGAGGGAAT
Glu Gly Ser Pro Leu Thr Gln Asp Asp Arg Gln Val Glu Leu Pro Val

      1827       1837       1847       1857       1867       1877       1887
TCTAAGCTGG ACGCACAGAA CAGTCTCTCC GTGGGAGGAG ACATTATGGG GCGTCCACCA CCACCCCTCC

      1897       1907       1917       1927       1937       1947       1957
CTGGCCATCC TCCTGGAATG TGGTCTGCCC TCCACCAGAG CTCCTGCCTG CCAGGACTGG ACCAGAGCAG

      1967       1977       1987       1997       2007       2017       2027
CCAGGCTGGG GCCCCTCTGT CTCAACCCGC AGACCCTTGA CTGAATGAGA GAGGCCAGAG GATGCTCCCC
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Figure 1 (Con't)

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      2037       2047       2057       2067       2077       2087       2097
ATGCTGCCAC TATTTATTGT GAGCCCTGGA GGCTCCCATG TGCTTGAGGA AGGCTGGTGA GCCCGGCTCA

      2107       2117       2127       2137       2147       2157       2167
GGACCCTCTT CCCTCAGGGG CTGCACCCTC CTCTCACTCC CTTCCATGCC GGAACCCAGG CCAGGGACCC

      2177       2187       2197       2207       2217       2227       2237
ACCGGCCTGT GGTTTGTGGG AAAGCAGGGT GGACGCTGAG GAGTGAAAGA ACCCTGCACC CAGAGGGCCT

      2247       2257       2267       2277       2287       2297       2307
GCCTGGTGCC AAGGTATCCC AGCCTGGACA GGCATGGACC TGTCTCCAGA GAGAGGAGCC TGAAGTTCGT

      2317       2327       2337       2347       2357       2367       2377
GGGGCGGGAC AGCGTCGGCC TGATTTCCCG TAAAGGTGTG CAGCCTGAGA GACGGGAAGA GGAGGCCTCT

      2387       2397       2407       2417       2427       2437       2447
GGACCTGCTG GTCTGCACTG ACAGCCTGAA GGGTCTACAC CCTCGGCTCA CCTAAGTGCC CTGTGCTGGT

      2457       2467       2477       2487       2497       2507       2517
TGCCAGGCGC AGAGGGGAGG CCAGCCCTGC CCTCAGGACC TGCCTGACCT GCCAGTGATG CCAAGAGGGG

      2527       2537       2547       2557       2567       2577       2587
GATCAAGCAC TGGCCTCTGC CCCTCCTCCT TCCAGCACCT GCCAGAGCTT CTCCAGGAGG CCAAGCAGAG

      2597       2607       2617       2627       2637       2647       2657
GCTCCCCTCA TGAAGGAAGC CATTGCACTG TGAACACTGT ACCTGCCTGC TGAACAGCCT GCCCCCGTCC

      2667       2677       2687       2697       2707       2717       2727
ATCCATGAGC CAGCATCCGT CCGTCCTCCA CTCTCCAGCC TCTCCCCAGC CTCCTGCACT GAGCTGGCCT

      2737       2747       2757       2767       2777       2787       2797
CACCAGTCGA CTGAGGGAGC CCCTCAGCCC TGACCTTCTC CTGACCTGGC CTTTGACTCC CCGGAGTGGA

      2807       2817       2827       2837       2847       2857       2867
GTGGGGTGGG AGAACCTCCT GGGCCGCCAG CCAGAGCCGG TCTTTAGGCT GTGTTGTTCG CCCAGGTTTC

      2877       2887       2897       2907       2917       2927       2937
TGCATCTTGC ACTTTGACAT TCCCAAGAGG GAAGGACTTA GTGGGAGAGA GCAAGGGAGG GGAGGGCACA
```

Figure 1 (Con't)

2947	2957	2967	2977	2987	2997	3007
GACAGAGAGG	CTACAGGGCG	AGCTCTGACT	GAAGATGGGC	CTTTGAAATA	TAGGTATGCA	CCTGAGGTTG
3017	3027	3037	3047	3057	3067	3077
GGGGAGGGTC	TGCACTCCCA	AACCCCAGCG	CAGTGTCCTT	TCCCTGCTGC	CGACAGGAAC	CTGGGGCTGA
3087	3097	3107	3117	3127	3137	3147
GCAGGTTATC	CCTGTCAGGA	GCCCTGGACT	GGGCTGCATC	TCAGCCCCAC	CTGCATGGTA	TCCAGCTCCC
3157	3167	3177	3187	3197	3207	3217
ATCCACTTCT	CACCCTTCTT	TCCTCCTGAC	CTTGGTCAGC	AGTGATGACC	TCCAACTCTC	ACCCACCCCC
3227	3237	3247	3257	3267	3277	3287
TCTACCATCA	CCTCTAACCA	GGCAAGCCAG	GGTGGGAGAG	CAATCAGGAG	AGCCAGGCCT	CAGCTTCCAA
3297	3307	3317	3327	3337	3347	3357
TGCCTGGAGG	GCCTCCACTT	TGTGGCCAGC	CTGTGGTGGT	GGCTCTGAGG	CCTAGGCAAC	GAGCGACAGG
3367	3377	3387	3397	3407	3417	3427
GCTGCCAGTT	GCCCCTGGGT	TCCTTTGTGC	TGCTGTGTGC	CTCCTCTCCT	GCCGCCCTTT	GTCCTCCGCT
3437	3447	3457	3467	3477	3487	3497
AAGAGACCCT	GCCCTACCTG	GCCGCTGGGC	CCCGTGACTT	TCCCTTCCTG	CCCAGGAAAG	TGAGGGTCGG
3507	3517	3527	3537	3547	3557	3567
CTGGCCCCAC	CTTCCCTGTC	CTGATGCCGA	CAGCTTAGGG	AAGGGCAGTG	AACTTGCATA	TGGGGCTTAG
3577	3587	3597	3607	3617	3627	3637
CCTTCTAGTC	ACAGCCTCTA	TATTTGATGC	TAGAAAACAC	ATATTTTTAA	ATGGAAGAAA	AATAAAAAGG
3647	3657	3667	3677	3687	3697	3707
CATTCCCCCT	TCATCCCCCT	ACCTTAAACA	TATAATATTT	TAAAGGTCAA	AAAAGCAATC	CAACCCACTG
3717	3727	3737	3747	3757	3767	3777
CAGAAGCTCT	TTTTGAGCAC	TTGGTGGCAT	CAGAGCAGGA	GGAGCCCCAG	AGCCACCTCT	GGTGTCCCCC
3787	3797	3807	3817	3827	3837	3847
CAGGCTACCT	GCTCAGGAAC	CCCTTCTGTT	CTCTGAGAAG	TCAAGAGAGG	ACATTGGCTC	ACGCACTGTG

Figure 1 (Con't)

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      3857       3867       3877       3887       3897       3907       3917
AGATTTTGTT TTTATACTTG GAAGTGGTGA ATTATTTTAT ATAAAGTCAT TTAAATATCT ATTTAAAAGA

      3927       3937       3947       3957       3967       3977
TAGGAAGCTG CTTATATATT TAATAATAAA AGAAGTGCAC AAGCTGCCGT TGACGTAGCT CGAG
```

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/03697

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[4] A61K 37/02, 31/705; C07K 17/06; C07H 15/24
U.S. CL 514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405, 406

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405,406

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Computer Search on CAS and Dialog; Files CA, Biosis, 155, 350, 351; For: CSF and (conjugate or link or complex or couple) and (toxin or cytotoxic agent or anthracycline)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US A, 4,504,586, (Nicolson), March 12, 1985, See Columns 1-2	1-8
Y	Science, Vol. 236, Issued June 1987; "The Human Hematopoietic Colony-Stimulating Factors", (Clark), pages 1229-37, See pages 1235-36.	1-8
Y	Blood, Vol. 67, Issued February 1986, "The Molecular Biology and Functions of the Granulocyte - Macrophage Colony - Stimulating Factors", (Metcalf), pages 257-67, See pages 259, 262-64.	1-8
Y	US, A, 4,675,382, (Murphy), June 23, 1987 See Columns 1-3.	1-2, 8-9
Y	Pharmac. Ther., Vol. 15, Issued 1982, "Chimeric Toxins", (Olsnes), pages 355-79, See pages 355, 357-62, 366.	1-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 January 1989	08 MAR 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Garnette D. Draper Garnette D. Draper

International Application No.
PCT/US88/03697

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Exp. Clin. Cancer Res. Vol. 3, Issued 1984, "Biochemical Aspects of Antibody - Directed Delivery of Toxins and Drugs to Target Cancer Cells, (Chersi), pages 217-23.	1-8
Y	Monoclonal Antibodies '84: Biological and Clinical Applications, Issued 1985, "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review" (Thorpe), pages 475-506.	1-8